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Exhibit 2

TABLE 9. *Pathogenetic mechanisms of autoantibodies*

Mechanism	Example
Complement-dependent lysis	Paroxysmal cold hemoglobinuria
Opsonization	Immune thrombocytopenia
Immune complexes	Systemic lupus erythematosus
Receptor inhibition	Myasthenia gravis
Receptor stimulation	Thyrotoxicosis
Inhibition of physiological peptide	Pernicious anemia

only rarely with autoantibodies. Paroxysmal cold hemoglobinuria, due to complement-fixing IgM autoantibodies, is the classic example of this mechanism. The autoantibodies in most other kinds of autoimmune hemolytic anemia do not fix complement efficiently because the sparse distribution of the autoantigen on the erythrocyte membrane does not allow cross-linking of adjacent IgG molecules by C1q. In those cases, the autoantibodies *opsonize* the cells, thereby facilitating their phagocytosis.

The glomerulonephritis of serum sickness—a response to exogenous antigens—exemplifies the kind of inflammation that *immune complexes* incite when they become deposited in tissues. Autoantibodies can also form immune complexes, by combining with autoantigens either *in situ* or in the circulation. Soluble immune complexes capable of circulating in the blood are formed by IgG antibodies when the ratio of antigen to antibody is high. These complexes are removed only slowly, unlike the large, rapidly phagocytosed aggregates that form when the antigen/antibody ratio is low. The soluble complexes are distributed throughout the body by the circulation and ultimately lodge in tissues with large filtering surfaces such as the kidneys and skin. Once deposited, the immune complexes activate the complement cascade, which in turn attracts inflammatory cells to the region (320). Complement-fixing cationic IgG antibodies are preferentially deposited in glomeruli, and they play a major role in generating nephritic lesions (321–325). Renal glomeruli have a net negative charge due to anionic proteoglycans in the basement membrane and epithelial foot processes (326,327). Therefore cationic immune complexes, with a net positive charge, tend to deposit and persist there (328–330).

Autoantibodies that bind to cell surface receptors can either inhibit or stimulate the specialized function of the cell without destroying it. In myasthenia gravis, autoantibodies bind to acetylcholine receptors on the postsynaptic membranes of muscles. The cross-linked receptors are internalized, causing a reduction in the number of exposed receptors and thus failure of the muscle to respond to acetylcholine released by motor nerve endings (331) (Fig. 8). An insulin-resistant form of diabetes mellitus is associated with autoantibodies to the insulin receptor; the number of insulin receptors on the cell membrane is normal but the autoantibodies block the action of insulin by binding to its receptors (332). The opposite situation applies to Graves' disease (thyrotoxicosis), in which there are autoantibodies that bind to receptors for thyroid stimulating hormone (TSH). When injected into

rats, anti-TSH receptor antibodies stimulate overproduction of thyroid hormones (333). The anti-TSH receptor autoantibodies cross-link the receptors, thereby falsely informing the cell that it is being stimulated by TSH (333). Still other autoantibodies can bind to a ligand and block its physiological activity. This occurs in pernicious anemia. Autoantibodies that bind intrinsic factor, a gastric peptide necessary for vitamin B_{12} absorption, prevent intestinal absorption of this essential vitamin for erythropoiesis (334).

IgG autoantibodies are notably important in the pathogenesis of the lesions of many autoimmune diseases. However, autoantibodies with other immunoglobulin isotypes can also be pathogenic. The pathological relevance of IgM autoantibodies is clear in the hemolytic anemia of cold agglutinin disease (335), in the peripheral neuropathy caused by anti-nerve autoantibodies (129,336), and in the vasculitis due to self-associating IgM cryoglobulins (337).

Representative Autoimmune Diseases and their Animal Models

Systemic Lupus Erythematosus

The human disease

The principal clinical manifestations of SLE, the prototypic systemic autoimmune disease, are arthritis, rash, and glomerulonephritis. Autoimmune thrombocytopenia, hemolytic anemia, and involvement of the central nervous system are common complications. About 90% of patients with the disease are young women; the median age at the time of diagnosis is 29. This marked preponderance of females is not seen before puberty or after the menopause, a reflection of the important influence of estrogens in SLE. The disease is highly variable; indeed it seems to consist more of a collection of syndromes than of a disorder with a uniform clinical pattern. In some cases the disease is violent and rapidly fatal despite intensive therapy. At the opposite end of the lupus spectrum are patients with only minor symptoms, whose longevity is unaffected. The activity of the disease can fluctuate: long quiescent periods of good health can terminate abruptly and inexplicably with the explosive onset of a new attack. The same lesions tend to recur in a given patient, and identical manifestations usually occur in identical twins with the disease.

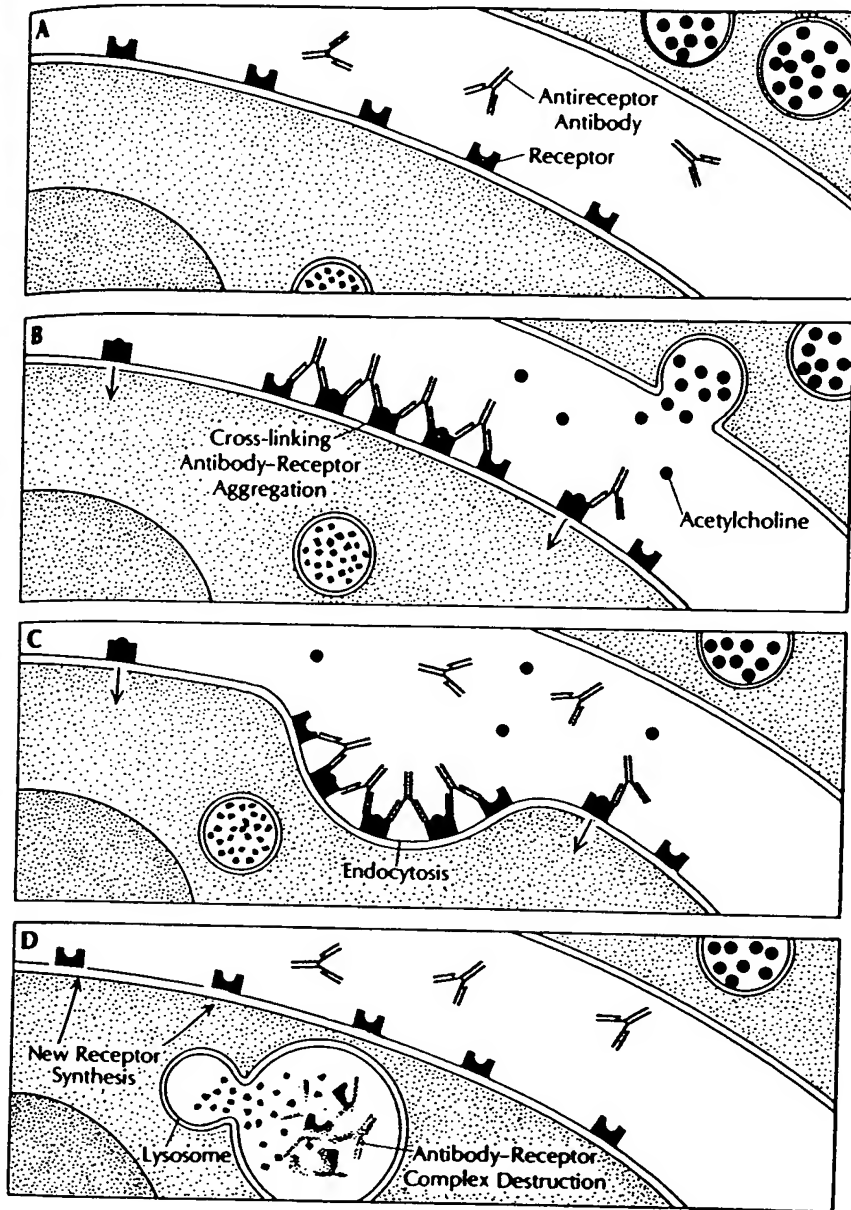An apparently large number of different autoantibodies

FIG. 8. Immunological mechanism in myasthenia gravis. Autoantibodies not only block but also cross-link acetylcholine receptors; the autoantibody–receptor complexes are endocytosed and degraded by lysosomal enzymes. New receptors formed on the cell surface undergo the same fate. (From Stobo, ref. 456, with permission.)

occur in lupus (Tables 5 and 8). The characteristic lupus autoantibody binds to DNA, but antibodies against ribonucleoproteins (e.g., RNP and Sm), histones, RNA, and nucleolar constituents are also produced. Antibodies against denatured DNA (single-stranded DNA) are not confined to SLE. However, autoantibodies against native, double-stranded DNA are highly specific for the disease. Another "marker" autoantibody in SLE, the anti-Sm antibody, binds to the protein component of a uridine-rich ribonucleoprotein (Table 8). It occurs in about 35% of lupus patients, but it has no known pathogenic role in the disease.

Studies of monoclonal autoantibodies produced by hybridomas derived from lupus patients have demonstrated that anti-DNA autoantibodies are often polyspecific; that is, they are capable of binding to both single-stranded DNA and double-stranded DNA, as well as to synthetic polynucleotides (338); they may also bind phospholipids

(339), cytoskeletal proteins (340), and other molecules (269). These cross-reactions may be due to a shared conformational determinant in the various antigens, one candidate being the sugar-phosphate backbone of DNA (Fig. 9). The polyspecificity of anti-DNA autoantibodies might account for some of lupus' protean manifestations. For example, an anti-DNA antibody that also binds to platelets could be responsible for the immune thrombocytopenia of the disease (338).

Before the onset of glomerulonephritis, there is often an increase in serum levels of IgG anti-DNA antibodies, particularly IgG_1 and IgG_3 antibodies, which are the dominant complement-fixing IgG subclasses in humans (341). These IgG anti-DNA antibodies form intermediate sized immune complexes by binding to DNA derived from the daily turnover and destruction of senescent cells. The immune complexes can deposit in basement membranes of renal glomeruli, dermal–epidermal junctions of the skin,
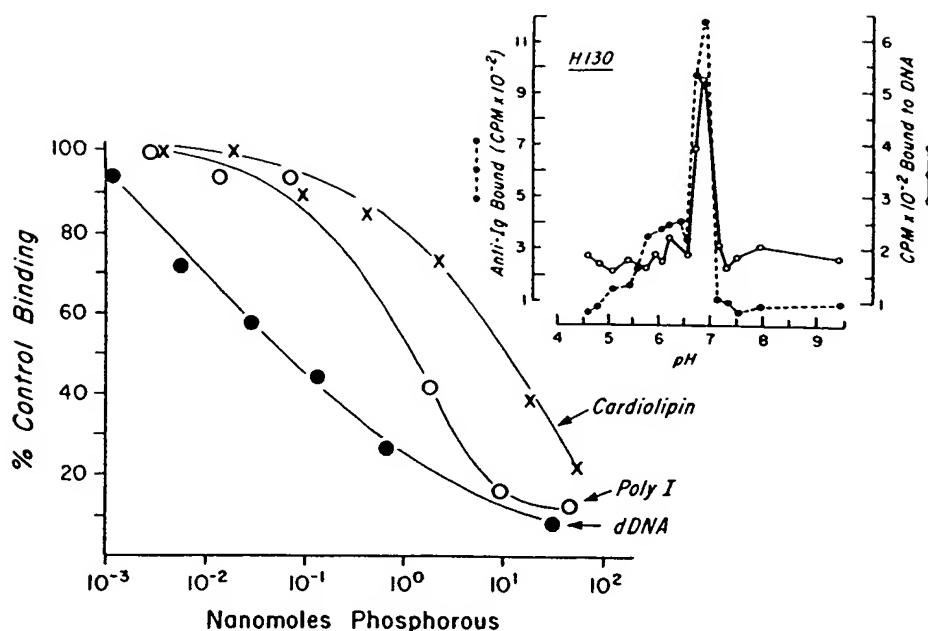
FIG. 9. Cross-reactions of a monoclonal anti-DNA antibody with the synthetic polynucleotide polyinosinic acid (Poly-I) and the phospholipid cardiolipin. Competitive inhibition of binding of the monoclonal autoantibody to DNA (100% control binding) by the three antigens is shown. Upper right: The polyreactive autoantibody is shown to be indeed monoclonal. (From Lafer et al., ref. 339, with permission.)

or in the choroid plexus. IgG anti-DNA antibodies in the glomerular deposits may be 1,000-fold more concentrated than in the serum (311,341). The renal deposits consist of granular accumulations of IgG and complement within the basement membranes of glomerular capillary tufts and under the capillary endothelium and epithelial foot processes (341). There, activation of the complement system evokes the complex events that culminate in inflammatory damage to the basement membrane. Consumption of complement components during the active phase of disease lowers the complement activity in serum (Fig. 10).

Lupus patients may also produce IgM and IgG rheumatoid factors that form cold-precipitable complexes (cryoglobulins) with IgG, including IgG anti-DNA antibodies. These aggregates can inflame peripheral blood vessels, which tend to be cooler than central blood vessels. Autoantibodies to red blood cells, platelets, and lymphocytes also occur, and they result in autoimmune hemolytic anemia, thrombocytopenia, and lymphopenia, respectively. The lymphocytotoxic antibodies of lupus can bind to the suppressor-inducer subset of $CD4^+$, $2H4^+$ T cells (204,205). We have already seen that depletion of these cells may contribute to the pathogenesis of SLE.

Genetic factors are important in susceptibility to lupus. The concordance of the disease in identical twins in high—over 65% (341). MHC-linked immune response genes and as yet unidentified disease-susceptibility genes play a role. HLA-DR2 or HLA-DR3 haplotypes are associated with a relative risk of SLE of about 3 and the risk is almost doubled when both haplotypes are simultaneously inherited (341). Another genetic factor has been mapped to the MHC class III region that encodes com-
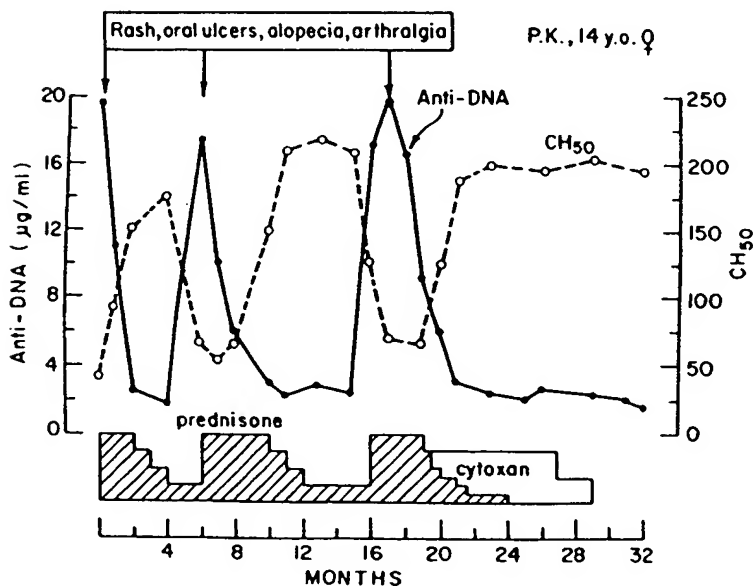

FIG. 10. Clinical course of a patient with systemic lupus erythematosus. Note the "mirror image" relationship between serum levels of anti-DNA antibodies and total hemolytic complement (CH_{50}). The reduced complement activity is due to consumption of complement components by tissue deposits of antigen—antibody complexes. Note the correlation between exacerbations of the disease (vertical arrows) and increased levels of anti-DNA antibodies.

TABLE 10. *Immune complex diseases and deficiency of complement components*

Deficient protein	Incidence
Clq, Clr, Cls	19/22 (87%)
C4	14/16 (88%)
C2	46/77 (60%)
C3	11/14 (79%)
C5, C6, C7, C8	10/99 (10%)
Properdin	0/12
Factor D	0/2
C1 inhibitor	Rare
C3b inactivator	⅙
β-1-H	½

From Schifferili et al., ref. 457, with permission.

plement components (Table 10). Deficiency of C4A (C4A "null" phenotype) occurs in approximately 11% of white SLE patients, compared with an incidence of approximately 1% in the normal population (342). The C4A null phenotype, usually due to deletion of the C4A gene, is found associated with the HLA-B8/DR3 haplotype in 50% of white SLE patients, whereas HLA-DR2 is found in 50% of patients without the deletion of C4A (342). Because C4A plays a critical role in eliminating immune complexes, its deficiency may contribute to the pathogenesis of SLE (342). It is worth pointing out here that the association between a particular MHC haplotype and an autoimmune disease is not absolute. A significant proportion of lupus patients does not have the HLA-DR2 (or HLA-DR3) haplotype, and conversely many individuals with that haplotype do not develop SLE. Thus a particular HLA haplotype itself may not determine the development of disease, but other susceptibility genes in linkage disequilibrium with it may be the primary contributors.

Animal models of SLE

Genetically uniform, inbred mouse strains that spontaneously develop a disease similar to human SLE have become essential tools in lupus research. The lupus mouse strains were not derived by any deliberate breeding protocols aimed at developing autoimmune animals. On the contrary, they all arose by chance. In addition to typical immunopathologic lesions of human SLE, these mice also have numerous immunologic abnormalities which may be either the cause of the disease or its consequence—or they may be irrelevant to lupus. Thus studies of these mice have substantially helped to understand the pathogenesis of SLE, but the etiology of the disease remains as complex in mice as it is in humans.

NZB mice and their F_1 hybrids

Among the murine models of SLE that have been investigated extensively, the New Zealand strains were derived first, during the late 1950s (23). These mice were

originally bred for different coat colors from mice of unknown genetic backgrounds. The objective was cancer research, but to the investigators' surprise the New Zealand black (NZB) strain developed autoimmune hemolytic anemia (23,343). When NZB mice were crossed with the New Zealand White (NZW) strain, the disease in the (NZB × NZW) F_1 progeny (B/WF$_1$) was severe lupus glomerulonephritis (23,343). A similar shift to lethal nephritis occurs when NZB mice are crossed with SWR mice (282,344–346). However, when NZB mice are crossed with other normal mouse strains—C57BL/6 or AKR, for example—there is no autoimmune disease in the F_1 progeny (Fig. 11). This shows that genes inherited from the "normal" parent strongly influence the expression of autoimmunity in NZB crosses (343,344). Certain differences from B/WF$_1$ crosses make NZB × SWR (SNF$_1$) crosses more suitable for a genetic analysis of autoimmune disease. NZW mice are not normal; they produce anti-DNA autoantibodies and develop nephritis late in life (347). By contrast, SWR mice are free of autoimmune disease and autoantibodies (344–346). SNF$_1$ mice therefore permit identification of the factors that a normal mouse genome contributes to the development of lupus nephritis (172,323). Both of these F_1 hybrids develop SLE spontaneously, without the superimposition of any lupus-accelerating genes, in contrast to the MRL and BXSB strains to be described below. Thus they permit a genetic dissection of various factors that may *primarily* be involved in the development of autoimmune disease.
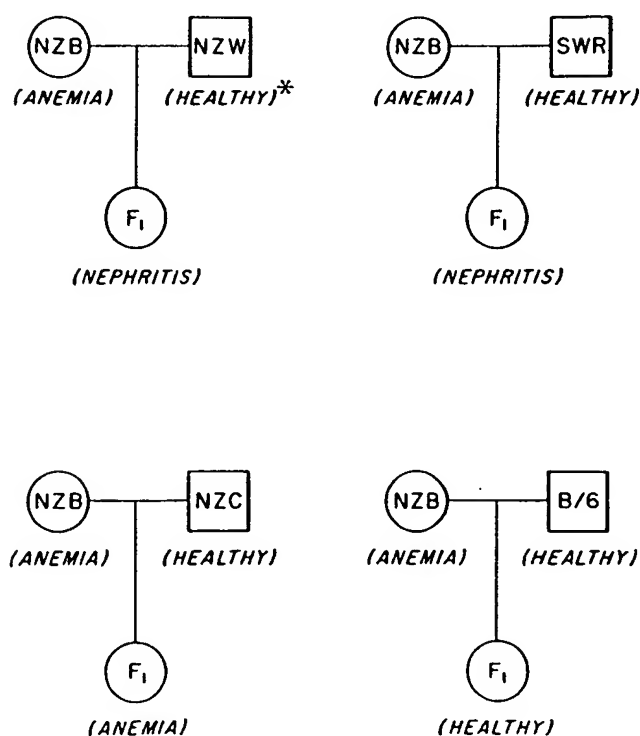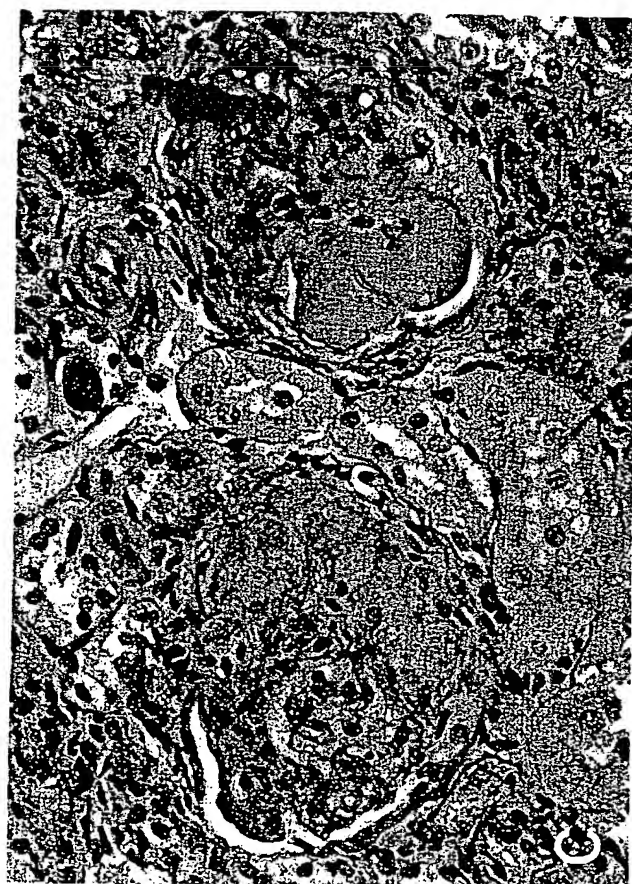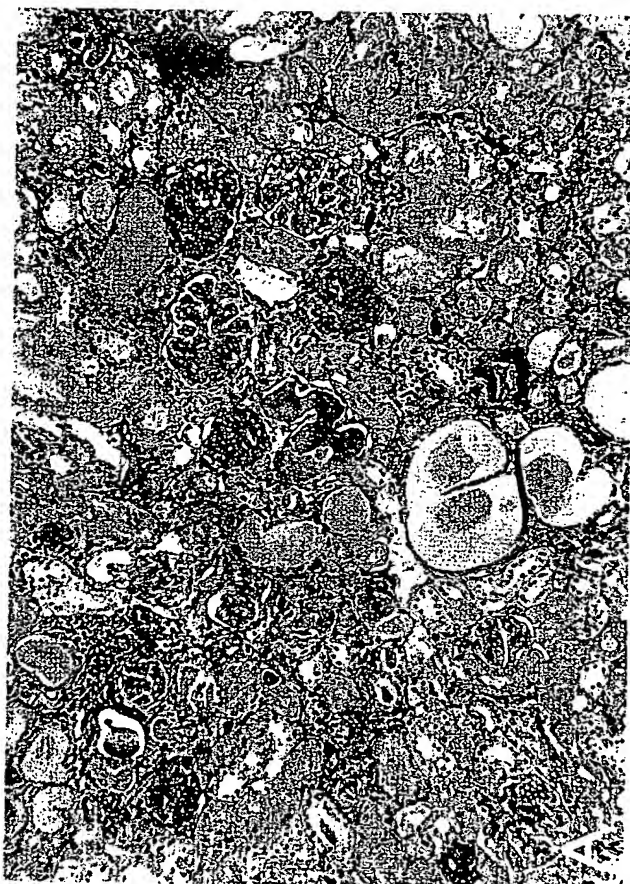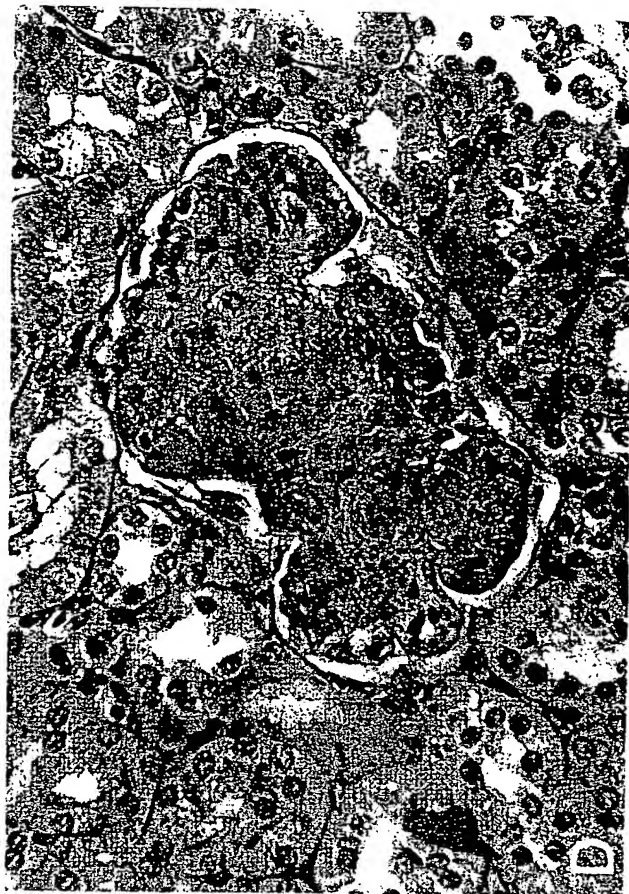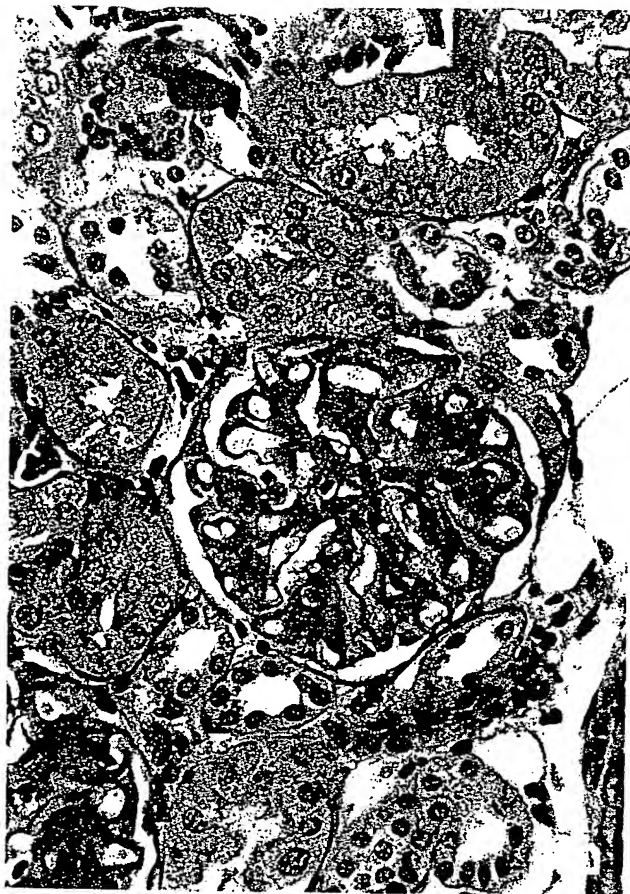

FIG. 11. The genetic influence of various normal mouse strains on the development of autoimmune disease in NZB hybrids. *The NZW strain is healthy when young but develops autoantibodies and nephritis when old.

The most important pathologic lesion in B/WF₁ and SNF₁ mice is glomerulonephritis (Fig. 12). Anti-DNA antibodies play a major role in this lesion. IgG anti-DNA antibodies deposited in the glomeruli are 6- to 30-fold more concentrated than in serum. Although anti-double-stranded DNA antibodies are considered to be the hallmarks of human lupus nephritis, most of the anti-DNA antibodies in the murine renal lesion bind to single-stranded DNA (324,348). Before the onset of nephritis, the production of anti-DNA antibodies shifts markedly from IgM to IgG isotypes (349). This switch is also associated with an increased cationic charge of the anti-DNA antibody population (189). The majority of the antibodies in the renal lesions are of the IgG_{2a} and IgG_{2b} isotypes, which are efficient complement-fixing subclasses (324,328). In SNF₁ mice, the IgH allotypes of the SWR and NZB parents are equally represented among the cationic anti-DNA antibodies. But these autoantibodies are absent from parental sera. Thus pathogenic anti-DNA autoantibodies that are dormant both in the SWR and in the autoimmune NZB parents become expressed in the SNF₁ progeny (324).

In addition to anti-DNA antibodies, antibodies to gp70 (a 70-kd glycoprotein related to the envelope protein of endogenous xenotropic retroviruses) are deposited in the renal lesions of some lupus strains (350). However, gp70 is not an essential antigen for the development of nephritis (345). Anti-DNA and anti-gp70 antibodies do not account for all the antibodies deposited in the renal lesions of lupus mice; the antigenic specificities of a substantial proportion of the renal immunoglobulin deposits are not known. The role of other autoantibodies, such as those against ribonucleoproteins and histones, in the development of nephritis is not clear.

The major manifestation of autoimmune disease in the NZB strain itself is hemolytic anemia (23,343). Anti-erythrocyte autoantibodies appear at 3 months of age; by the age of 15 months, more than 90% of NZB mice of both sexes produce them. The anti-erythrocyte autoantibodies are of two types: anti-X reacts with an exposed glycoprotein on the surface of red cells and is responsible for the anemia; anti-HB, also present in normal mouse strains, binds to a cryptic antigen that can be exposed by bromelein. The anti-X autoantibodies are of the IgG class and their production is T cell dependent (351,352). The nonpathogenic anti-HB antibodies, produced by Ly-1 B cells, are of the IgM isotype and their V region sequences are highly conserved (147,157). The incidence of nephritis in NZB mice is low, occurring usually in the second year of life, and the lesions themselves are rarely severe. Most NZB anti-DNA antibodies are of the IgM class and the low levels of IgG anti-DNA antibodies that they produce are usually anionic or neutral in charge (189,323).

These properties of NZB anti-DNA antibodies can account for the difference in the diseases developed by the NZB strain and its SNF₁ and B/WF₁ hybrids (322).

MRL mice

Two congenic MRL strains have been developed (353). One, MRL-*lpr/lpr*, develops massive lymphadenopathy and severe lupus. The lymph node enlargement is determined by an autosomal recessive gene for lymphoproliferation (*lpr*). The other strain, without lymphadenopathy, is termed MRL-+/+. Its genome, virtually identical to that of the MRL-*lpr/lpr* strain, lacks the *lpr* genes. MRL-+/+ mice develop a mild form of lupus nephritis late in life, whereas the severe nephritis of MRL-*lpr/lpr* mice has an early onset. The MRL strain arose by accident from a series of crosses that were meant to transfer a mutation for achondroplasia (this defect has no relation to lupus) from the high-leukemia AKR mouse strain to a low-leukemia strain. During the inbreeding, other congenital defects became manifest. To rescue the line, the mice were backcrossed to several other healthy inbred strains. Subsequent rigid inbreeding generated the MRL line. During the twelfth generation of inbreeding, some of the offspring developed massive generalized lymph node enlargement, whereas others did not. These two types of mice were separated and by further inbreeding they became two MRL sublines. Cross–intercross matings between those sublines led to the two congenic MRL strains, MRL-*lpr/lpr* and MRL-+/+. This complex process took 20 years (1960 to 1980)!

The *lpr* gene has been transferred to the genetic background of several normal inbred strains, including C57BL/6, BALB/c, C3H/HeJ, AKR, C57BL/10, and SJL. Although these congenic *lpr* strains produce autoantibodies, they do not develop the severe immunopathologic lesions of MRL-*lpr/lpr* mice. The *lpr* gene produces full-blown SLE only in the genetic background of its congenic partner, MRL-+/+ (354). Therefore the underlying or primary mechanism of autoimmunity in the MRL model lies in the MRL-+/+ background; the *lpr* gene acts by accelerating and increasing the severity of the disease.

BXSB mice

BXSB is a recombinant inbred line with 50% of its genome derived from each of the progenitor strains, a C57BL/6 female and a SB/Le male (353). These animals also emerged serendipitously; the progenitor strains had no apparent connection with lupus. Nevertheless, genes

FIG. 12. Representative renal lesions in SNF₁ mice. A: All glomeruli in the field are affected. Note the lymphocytes infiltrating the interstitial tissue. B: Glomeruli with prominent thickening of mesangium and capillary loops. C: Markedly thickened and sclerotic glomeruli. D: Extensive distortion of normal glomerular architecture by immune deposits. (From Eastcott et al., ref. 346, with permission.)

linked to the Y chromosome of the BXSB strain that were inherited from the SB/Le background markedly accelerate autoimmune disease in these mice. The Y-chromosome-linked autoimmune accelerator (*Yaa*) gene of BXSB mice can accelerate lupus only if other autosomal genes from the SB/Le background are present (355).

Of these various strains, MRL-*lpr/lpr* mice, both males and females, develop severe disease at the earliest age (Fig. 13). Anti-DNA antibodies are detectable in these animals by 1 to 2 months. Death from lupus nephritis occurs between 3 and 6 months in 50% of these mice and almost all are dead by 9 months of age (348,353). By contrast, MRL-+/+ mice, both males and females, die from lupus nephritis between 11 and 24 months, reaching 50% mortality at about 17 months of age (348,353). Lupus is also markedly accelerated in BXSB male mice, with an age-related mortality similar to the MRL-*lpr/lpr* strain. However, the onset of the disease in BXSB females is delayed. In B/WF₁ and SNF₁ hybrids, death from severe lupus nephritis occurs between 5 and 12 months in the females, whereas in males the disease is milder and its onset is later (10 to 19 months of age) (343,344,346). The early onset and severity of lupus in the female NZB hybrids contrast sharply with the Y-chromosome-linked male effect in the BXSB model and is probably related to estrogenic hormones. However, a hormonal influence is not apparent in MRL-*lpr/lpr* mice.

Chronic graft-versus-host disease

A graft-versus-host reaction occurs when lymphocytes are injected into histoincompatible hosts that cannot reject the grafted cells (356–358). The reaction depends on T cells in the donor inoculum and on the extent of incompatibility between the graft and the recipient. The severest reactions occur with Class II MHC differences. In some cases the graft-versus-host reaction is acute, with rapid wasting (runting) of the animal and atrophy of lymphoid tissue. A chronic graft-versus-host reaction can be in-

duced in mice if 4- to 8-week-old F₁ hybrid animals are injected with lymphocytes from one of the parental strains (357,358).

The graft-versus-host reaction can produce autoimmune disorders. The first of these to be reported, immune hemolytic anemia, occurred in chickens that had been injected as 18-day embryos with spleen cells from adult donors. After hatching, the chicks developed severe anemia with antibodies coating their red cells (359). The features of the chronic graft-versus-host reaction in F₁ mice are remarkably similar to the autoimmune disease of NZB mice and their F₁ hybrids. Either autoimmune hemolytic anemia (360) or immune-complex nephritis can develop, depending on the donor–host combination (361). And, like NZB mice, some F₁ recipients also develop a persistent lymphoproliferative disease that culminates in malignant lymphoma (362). The renal lesion takes the form of membranous glomerulonephritis, with immunoglobulin deposits in the glomeruli (361). A Class II MHC difference between donor and host strongly favors the development of nephritis and lymphomas (361).

Although it might be thought that these abnormalities are due to immune reactions of the graft against the host, the actual situation is more interesting. Host B cells proliferate during the graft-versus-host reaction (363), and authentic autoantibodies, including anti-red cell (364) antinuclear (365), and anti-DNA antibodies (366), are produced. The recipient's helper T cells are unnecessary for the development of autoimmunity in the graft-versus-host reaction, but those of the donor are essential (358). When stimulated by the recipient's Class II MHC antigens, the donor's T cells cause polyclonal activation of the host's B cells: autoimmunization does not occur if the host is genetically deficient in B cells (367), and the autoantibodies have the allotypic markers of the host (366). Moreover, F₁ mice that develop autoimmune hemolytic anemia and nephritis after inoculation of parental spleen cells produce large amounts of a T cell lymphokine, IL-5, a B cell differentiation factor (190). This is the very lymphokine that cultured spleen cells of MRL-*lpr/lpr* mice produce in abundance.
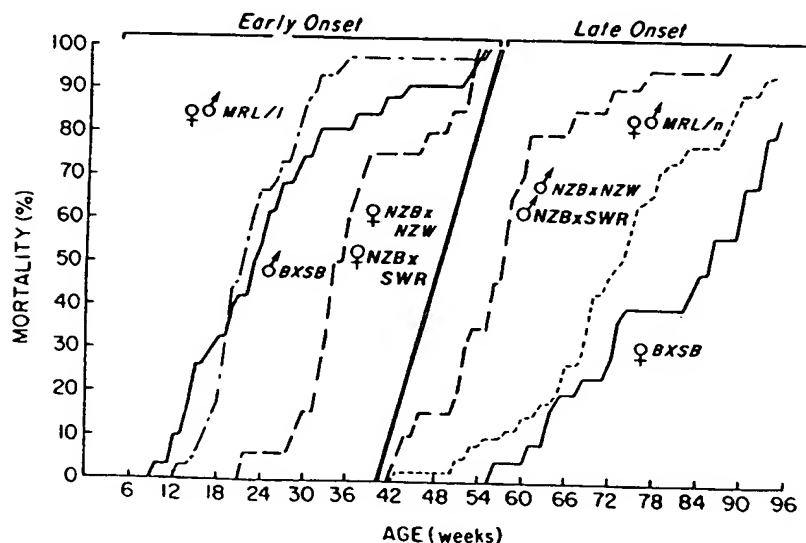

FIG. 13. Mortality patterns of mouse strains with early- and late-onset lupus. (Adapted from Theofilopoulos and Dixon, ref. 348.)

It is worth stressing that, unlike experimentally induced models of organ specific autoimmune diseases, no exogenous autoantigen is required in the graft-versus-host model of autoimmunization. Nor is it likely that release of intracellular autoantigens plays a role because severe nephritis can develop in the absence of any of the tissue-destructive manifestations of a graft-versus-host reaction (368). In fact, destruction of host tissues actually impairs the autoimmune process, as shown by the following. Injection of DBA/2 T cells into (C57BL/6 × DBA/2)F_1 recipients results in autoimmunization, whereas C57BL/6 T cells produce the opposite: profound immunodeficiency (369). In the latter combination, donor Lyt-2$^+$ cytotoxic T cells predominate, whereas in the first model DBA/2 L3T4$^+$ helper T cells determine the outcome because the DBA/2 parent has a defect in generating Lyt-2$^+$ cytotoxic T cells against class I antigens of the F_1 (369). Selective removal of Lyt-2$^+$ T cells from the C57BL/6 donor inoculum leads· to the same chronic immunostimulatory graft-versus-host reaction as in the DBA/2 → F_1 combination (369). Moreover, injection of C57BL/6 cells into (C57BL/6 × C57BL/6.CH-2^{bm12})F_1 mice leads to autoimmunization; in this case the donor differs from the F_1 only by the mutation in Ia that results in activation of donor L3T4$^+$ T helper cells. Lyt-2$^+$ cytotoxic T cells from the donor are not induced due to class I MHC identity (368).

Autoimmunization in the chronic graft-versus-host reaction thus entails polyclonal B cell activation driven by polyclonally activated T cells and their lymphokines, the same mechanism we have discussed for NZB mice, their F_1 hybrids, and MRL-*lpr/lpr* mice. The particular manifestations of autoimmune disease in the graft-versus-host disease model—autoimmune hemolytic anemia or nephritis—are genetically controlled, most likely by genes outside the MHC. The broad picture that emerges from all these studies is that autoimmunization of the lupus type can occur when the normal preimmune B cell repertoire is triggered inappropriately by T cells.

Rheumatoid Arthritis

The human disease

Rheumatoid arthritis, a prevalent, chronic, and disabling disease, is a major representative of a large group of *rheumatic diseases*. These conditions include scleroderma, dermatomyositis, and polymyositis (Table 11). Rheumatoid arthritis occurs in both adults and children (juvenile rheumatoid arthritis). Its principal manifestation is arthritis, usually affecting many joints (polyarthritis) simultaneously or in sequence. In contrast to the *arthralgia* of SLE, which only rarely leads to crippling, the arthritis of rheumatoid arthritis can result in destruction of the joint with consequent deformity. The disease is not confined to joints. Vasculitis, caused by immune complexes, can involve the skin, the eye, and the lung.

The arthritis results from a complex interaction of synovial cells with various cellular elements (and their sol-

TABLE 11. *Rheumatic diseases with an autoimmune component*

Rheumatoid arthritis (inflammatory polyarticular arthritis)

Systemic lupus erythematosus (arthralgia, rash, nephritis)

Sjögren's syndrome (lacrymitis, parotitis, arthritis)

Scleroderma (fibrosis of skin and internal organs)

Mixed connective tissue disease (features of SLE and scleroderma)

Dermatomyositis (inflammation of striated muscle and skin)

Polymyositis (inflammation of striated muscle)

Reiter's syndrome (arthritis, uveitis, conjunctivitis, and rash)

Behçet's disease (recurrent mucous membrane ulcers and arthritis)

uble products) that infiltrate from the circulation into the synovial lining of joints (370). The actual cause of the joint lesion is unknown, but a plausible sequence of events is this. An unidentified agent or mechanism initiates an inflammatory response in the synovium. Small blood vessels are damaged and lymphocytes and monocytes accumulate in the perivascular space. CD4$^+$ helper T cells predominate in the initial inflammatory lesions. Macrophage-like phagocytic synovial lining cells and interdigitating synovial fibroblasts (synovial dendritic cells) proliferate and express abundant amounts of Class II MHC antigens. This activates the infiltrating helper T cells, leading them to express MHC antigens. The activated T helper cells seem to drive B cells infiltrating the synovium to produce immunoglobulins. The specificity of the majority of these locally synthesized antibodies is unknown, but some are IgG rheumatoid factors which bind to other IgG molecules in the joint to form immune complexes. The immune complexes activate the complement cascade, leading to increased vascular permeability, infiltration with more inflammatory cells, and an influx of neutrophils. By this time the joint is swollen, hot, and painful. The polymorphonuclear leukocytes and phagocytic synovial cells are stimulated after ingesting the immune complexes and they release various lysosomal proteases, free oxygen radicals, and various arachidonate metabolites, such as prostaglandins and other inflammatory products of the lipoxygenase pathway. All these soluble products damage collagen and the cartilage matrix of the joint. The macrophages are also activated by various lymphokines (γ interferon) produced by the activated T cells. The macrophages produce IL-1, which in turn stimulates snyovial dendritic cells and chondrocytes to proliferate and produce collagenases (in latent form) and plasminogen activators. The latter convert serum plasminogens that enter the inflamed joint into plasmin, which in turn activates the collagenases. Lysosomal proteases break down proteoglycans and collagen which form the matrix of cartilages, ligaments, and tendons of the joint. Thus the initial inflammatory infiltrates lead to proliferation of synovial cells, forming a chronic granulomatous lesion that in turn

invades and erodes cartilage and other components of the rheumatoid joint. It should be stressed that not all the steps in this complex sequence have been rigorously demonstrated.

Rheumatoid factors, autoantibodies against the Fc region of IgG molecules, are present in the serum of most adult patients with rheumatoid arthritis. By contrast, they occur in only 5 to 10% of patients with juvenile rheumatoid arthritis. The autoantibodies are usually of IgM and IgG class. Although IgM rheumatoid factor is routinely measured as an aid to diagnosis of the disease, it probably has no role in the joint lesions. However, IgM/IgG immune complexes can contribute to the systemic vasculitic complications of rheumatoid arthritis. Reduced glycosylation of the $C\gamma2$ region of serum IgG has been found in rheumatoid arthritis (371). This abnormality may be related to the reduced galactosyltransferase activity of B cells (but not of T cells and monocytes) from patients with the disease (372). Since the binding site for rheumatoid factor is in the $C\gamma2–C\gamma3$ junction domain of IgG, the abnormally low galactosylation of IgG in that region (Fig. 14) could render the molecule immunogenic (371).

IgM rheumatoid factor is normally produced during immune responses to exogenous antigens (373), and IgM and IgG rheumatoid factors are often increased in the serum of patients with other diseases, especially chronic bacterial or parasitic infections, without the development of arthritis. In those cases, however, the rheumatoid factors do not seem to be produced within joints, as they are in rheumatoid arthritis. Patients with rheumatoid arthritis may also produce other autoantibodies, including DNA and histones, but they do not often develop signs of SLE.

Susceptibility to rheumatoid arthritis is linked to the HLA-DR4 haplotype, but the association is not absolute; most DR4$^+$ people are healthy. Moreover, only 75% of caucasian patients with rheumatoid arthritis are DR4$^+$,

the remaining 25% are DR4$^-$, most of them being DR1$^+$; the incidence of DR4$^-$ rheumatoid arthritis is even more prominent in other ethnic groups. Two-dimensional peptide maps of HLA-D region gene products, analysis of restriction fragment length polymorphism (RFLP) of HLA-D region genes, typing of HLA-D region products by T cells clones, and sequences of the HLA-D region genes have clarified the issue. Irrespective of the serologic HLA-DR type (e.g., DR4 or DR1), a stretch of amino acid sequences in the third hypervariable region of the β_1 domain of HLA-DR β chain molecules are homologous in different rheumatoid arthritis patients (374). Gene conversion events could have led to insertion of these homologous sequences in different HLA-DR molecules. Since this region of the N-terminal β_1 domain of the MHC Class II molecule participates in forming the antigen-binding cleft, these shared sequences may be crucial for generating the autoimmune response in rheumatoid arthritis (see section, The MHC and Autoimmunization).

T cells of HLA-DRw4-positive rheumatoid arthritis patients are high responders to collagen in vitro, but so are T cells from HLA-DRw4$^+$ normal subjects (375). The collagen epitope appears to be on a $(Gly-Pro)_n$ peptide determinant which is exposed only after degradation of the molecule. The positive response of HLA-DRw4 people seems to reflect a lack of suppressor T cells (376). Many patients with rheumatoid arthritis also produce antibodies to denatured collagen, probably as a consequence of the disease, but some patients also have high levels of antibodies to native Type II collagen, a component of the hyaline cartilage of joints (377).

Animal models of rheumatoid arthritis

In addition to nephritis, MRL-*lpr/lpr* mice also develop a form of arthritis that resembles the human disease (348). The joint lesions begin to appear at about 2 months of age and 3 to 4 months later 75% of the mice have arthritis. The animals produce substantial amounts of IgM and IgG rheumatoid factors, which form soluble immune complexes by binding to the $C\gamma3$ domain of IgG. The pathogenic role of the IgM rheumatoid factors in MRL-*lpr/lpr* mice is unclear; C3H-*lpr/lpr* and C57BL/6-*lpr/lpr* mice do not develop arthritis despite higher levels of serum IgM rheumatoid factors than those in MRL-*lpr/lpr* mice (348). 129-SV mice and other strains, when injected with lipopolysaccharide or antigen–antibody complexes, also produce substantial amounts of rheumatoid factor without developing arthritis (373,378,379). In normal mouse strains the rheumatoid factors are heterogeneous, whereas some rheumatoid factors in the *lpr* strains (both the arthritic MRL-*lpr/lpr* and the nonarthritic C3H-*lpr/lpr*) are oligoclonal (163).

Two other forms of experimental arthritis, *autoimmune collagen arthritis* and *adjuvant arthritis*, can be induced in certain strains of rats and mice. Neither of these types of experimental arthritis has the systemic features of human rheumatoid arthritis, nor is there production of rheumatoid factors. Immunization of rats or mice with
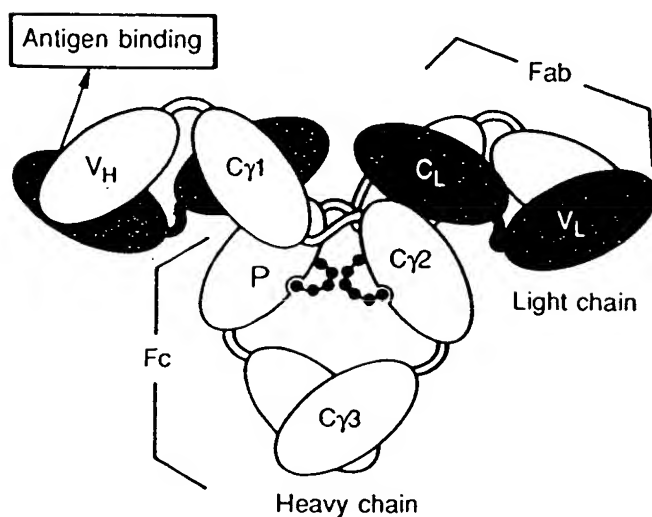

FIG. 14. Glycosylation of human IgG. The two $C\gamma2$ domains in the Fc portion of the molecule are held apart by a bridge formed by two oligosaccharides (●-●-●) which bind to a lectinlike pocket (P) on the surface of the $C\gamma2$ domains. (From Axford et al., ref. 372, with permission.)

heterologous type II collagen induces an inflammatory polyarthritis within 10 to 20 days (377). Only native collagen (prevalent in joint cartilage) can induce the disease, and no mycobacterial or other adjuvants are necessary. The initiating events are not completely understood, but complement-fixing IgG antibodies against collagen deposit in the articular cartilage; anti-type II collagen antibodies are concentrated 15-fold in arthritic joint fluids, as compared to serum. A transient form of the disease can be induced by IgG anti-type II collagen antibodies; a more persistent and severe disease occurs on transfer of collagen-specific
T cells (377,380). Susceptibility to collagen-induce arthritis is linked to MHC loci: RT-1 in rats and H-2 in mice. In the latter, strains with the H-2^q haplotype (B10.Q, B10.G, and DBA/1J) are highly susceptible to arthritis after immunization with native type II chicken collagen and they produce high levels of IgG2a antibodies to collagen. Susceptibility has been mapped to the I-A^q subregion (381). SWR mice, which also have the H-2^q haplotype, do not develop arthritis, but they are deficient in C5. They may also lack certain Vβ T cell receptor genes utilized by collagen specific T helper cells (382).

By contrast with the synergistic effects of antibodies and T cells in autoimmune collagen arthritis, adjuvant arthritis is a purely T cell-mediated autoimmune disease. This form of arthritis can be induced in susceptible strains of rats (e.g., Lewis rats) by an injection of *Mycobacterium tuberculosis* in oil (complete Freund's adjuvant). There is a structural resemblance between mycobacterial peptidoglycans and proteoglycans in joint cartilage; indeed, a nonapeptide consisting of amino acid residues 180–188 of a *Mycobacterium tuberculosis* antigen contains the epitope recognized by T cells mediating adjuvant arthritis; this epitope also occurs in the link protein of cartilage proteoglycan. Moreover, T cells from patients with rheumatoid arthritis respond to this shared epitope (383). The principal histopathologic finding in adjuvant arthritis is granuloma formation in the joints. The lesion can be transferred to syngeneic rats by T cells from rats with the disease. These arthritogenic T lymphocytes need be activated only by concanavalin A (384). A helper T cell clone that can induce arthritis has also been derived from arthritic rats (312); this clone, A2, can induce arthritis only in irradiated rats. If attenuated by prior irradiation, the A2 clone "vaccinates" nonirradiated rats against adjuvant arthritis by inducing specific suppressor cells (312). A subline of A2 (A2b), also arthritogenic, cannot protect rats against adjuvant arthritis; it has the special property of responding *in vitro* not only to *M. tuberculosis* antigens but also to proteoglycans in cartilage (97)—an example of antigenic mimicry.

Diabetes Mellitus

The human disease

Diabetes mellitus is a disorder of glucose metabolism. The resulting hyperglycemia increases serum osmolarity, leading to great thirst and a marked increase in urine production. There are two principal forms of the disease: Type I diabetes, in which the patient depends on exogenous insulin to maintain normal glucose metabolism, and type II diabetes, in which insulin treatment is frequently unnecessary. Type I diabetes affects 0.2 to 0.5% of the population; the peak age of onset is 11 to 12 years of age (385). In the United States there are over 100,000 children with diabetes who require daily insulin therapy. Because of its characteristic (but not exclusive) occurrence in children, the disease is also referred to as juvenile diabetes. Type I diabetes is a serious disorder and can be fatal if untreated. Numerous complications can occur after years of diabetes, including diabetic retinopathy (a cause of blindness), renal failure, and accelerated arteriosclerosis.

Type I diabetes is believed to be an autoimmune disease in which the insulin-producing β cells of the islets of Langerhans in the pancreas are destroyed. Pancreas grafts from an identical nondiabetic twin can also undergo insulitis when transplanted into the diabetic twin (386), so whatever the inciting factor may be, it culminates in an immunological process that can injure ostensibly normal β cells. The specific lesion of the disease is called *insulitis* (Fig. 15), an infiltration of the islets of Langerhans with mononuclear cells, which consist mainly of activated HLA-DR$^+$ T cells of the cytotoxic/suppressor (CD8$^+$) variety, and to a lesser extent of CD4$^+$ cells (80). These anti-β cell T cells are probably MHC restricted (385,387).

Autoantibodies against islet cells (ICA), insulin, and also insulin receptors occur in Type I diabetes. They are detectable before the onset of the disease and, notable for the latter two autoantibodies, before any insulin therapy (56,388–392). The autoantibodies against insulin receptors may arise from the type of idiotypic network discussed in the section on autoantibody idiotypes. The antibodies against islet cells recognize a 64 kd β cell protein that is associated with gangliosides (56). Surveys of susceptible but nondiabetic children have disclosed that the presence of complement-fixing IgG antibodies against islet cells is highly predictive of the development of Type I diabetes (56,385,388). These kinds of ICA can mediate β cell destruction *in vitro* (385), but whether they are the primary cause of the lesion or whether they arise as a secondary consequence of β cell damage by T cells is unclear. ICA can also be found in 0.5% of normal people and in 6% of patients with other autoimmune endocrine diseases (56,385).

Other organ specific autoimmune diseases may occur in association with Type I diabetes. In these cases, involvement of one endocrine organ follows another by 5 to 10 years, suggesting an underlying genetic predisposition but not a common pathogenic autoantibody (393). On the contrary, the autoantibodies are specific for each organ and do not cross-react (393). Viral infections may be cofactors in the pathogenesis of the disorder. Antecedent infections, particularly with Coxsackie B-4, have been associated with the development of Type I diabetes.

Type I diabetes has a genetic basis, and multiple genes are involved. The risk of developing the disease if one parent has Type I diabetes, 8 to 10%, increases to about 25% if both parents have the disease (394). About 95% of
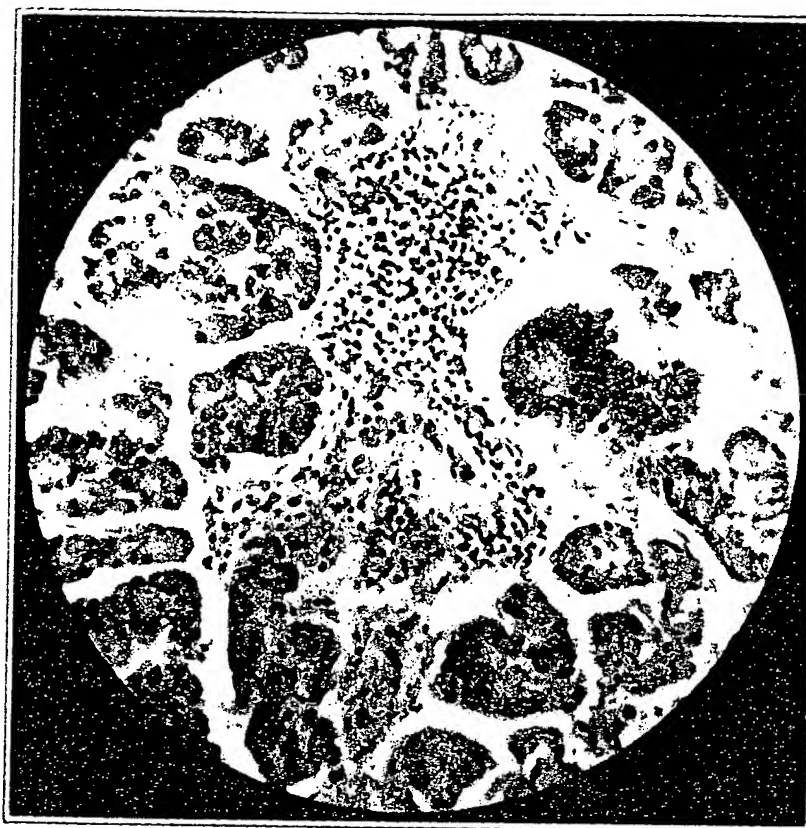
FIG. 15. Insulitis of a pancreatic islet from a 19-year-old man who died after 11 months of type I diabetes. The islet (center of photograph) is heavily infiltrated by lymphocytes. The uninvolved surrounding tissue consists of the acinar cells of the pancreas, which secrete digestive enzymes into the intestines. This classic photograph, originally published in *Pathology of Diabetes Mellitus* (Lee & Febiger, Philadelphia, 1930, p. 44) by Shields Warren, a pioneer in studies of diabetes, was generously provided by Dr. George Eisenbarth, Joslin Diabetes Center, Boston, MA.

caucasians with Type I diabetes have HLA-DR3 or HLA-DR4 haplotypes, or both, as compared to 40% of the general population (395). The effects of HLA-DR3 and HLA-DR4 may be additive: up to 50% of patients are DR3/DR4 heterozygotes, compared to 5% of the general population. In identical HLA-DR3/DR4 twins, concordance for Type I diabetes is 70%, in contrast to a 40% concordance if they have either DR3 or DR4, but not both (396). Indeed, the susceptibility to diabetes is actually linked to HLA-DQ genes which are in linkage disequilibrium with the HLA-DR genes mentioned above. Southern blot analysis with a gene probe for the β chain of HLA-DQ has identified a combination of RFLPs that are more closely associated with Type I diabetes than those determined by serologic DR typing (397). Two alleles of the DQβ locus, encoding the serologic specificities DQw3.1 and DQw3.2, have been identified. They have different amino acid residues at position 57, the polymorphic and functionally important region of the DQβ chain. Homozygosity of DQβ 3.2 alleles that do not have Asp at residue 57 occurs in 90% of caucasians with Type I diabetes, which explains the apparent recessive inheritance of MHC-linked susceptibility to the disease (397). Quite remarkably, an analogous MHC alteration occurs in the NOD mouse model of type I diabetes. The amino acid residue at position 57 may be important here because of its location in the antigen binding cleft of the MHC Class II molecule (397). Nevertheless, other genes in the MHC locus may also be involved. Only 2% of DQ Asp-57 negative individuals develop Type I diabetes, and in non-caucasians (or the diabetes-prone BB rat described below) diabetes usually oc-

curs even in the presence of ASP-57 (397). A T cell receptor β chain gene polymorphism has also been found to be associated with human Type I diabetes (398), suggesting that both heterozygous T cell receptor and HLA-DR genotypes confer susceptibility to the diseases, a situation analogous to the susceptibility to lupus nephritis in NZB × SWR mice (172).

Animal models of Type I diabetes

BB rats. The Bio-Breeding (BB) rat strain was developed from a commercial colony of Wistar rats at the Bio-Breeding Laboratories in Ottawa. The animals spontaneously develop a disease similar to Type I diabetes, with insulitis and autoantibodies against islet cells and insulin (56,385,399,400). The disease develops equally in animals reared in conventional and germ-free environments (385). The lymphocytic infiltrates in the islets consist of Ia+, IL-2 receptor+ T cells, and macrophages, but there are no B cells in the lesion (385,399). Insulitis can be prevented by treatment with anti-T cell antibodies, by antibodies to a nonvariant T cell receptor epitope, or by neonatal thymectomy (385). By selective breeding, a diabetes-resistant subline of BB rats that are histocompatible to the diabetes-prone rats was developed (385). The disease can be transferred to the diabetes-resistant strain with concanavalin-A-stimulated T cells from the diabetes-prone strain (385,401–403). Conversely, bone marrow transplantation or T lymphocyte transfusions

from resistant rats can prevent diabetes in diabetes-prone rats (385,401–403). Low-dose irradiation or cyclophosphamide markedly increases the incidence of type I diabetes in the diabetes-resistant line, implying a role for suppressor T cells. Such a regulatory T cell has indeed been identified in resistant rats, and administration of a monoclonal antibody that selectively depletes these cells triggers the development of diabetes (385,404). Thus the diabetes in BB rats is a T-cell-mediated disease; the balance between islet cell antigen specific T helper and suppressor cells determines susceptibility or resistance to the disease.

Multiple genes determine the development of diabetes in BB rats. One of them is linked to the rat MHC and another, not linked to the MHC, is associated with the T cell lymphopenia found in all diabetes-prone BB rats (385,405). The lymphopenia is not essential for the development of diabetes. Islets of Langerhans from diabetes-resistant rats are rapidly destroyed when transplanted to the diabetes-prone strain, suggesting that an abnormal β cell antigen is not essential for initiating insulitis (385).

NOD mice. The nonobese diabetic (NOD) mouse originated from a noninbred ICR strain. Insulitis in this animal begins between 5 and 8 weeks of age, and by 7 months 70% of females and 40% of males become diabetic. T cells transferred from diabetic mice to young nondiabetic NOD mice induce diabetes within 2 to 3 weeks (406). At least three functionally recessive genes (or gene clusters) determine the development of diabetes in NOD mice: one is linked to the H-2 region on chromosome 17; another has been localized proximal to *Thy-1/Alp-1* cluster on chromosome 9 (407). The linkage of diabetes to the MHC is interesting because NOD mice express the I-A but not the I-E gene product (408,409). NOD mice that do express I-E molecules, as a result of breeding with I-E-expressing transgenic C57B1/6 mice, do not develop insulitis (409). The expression of I-E molecules may induce suppressor T cells, thereby protecting the animals from diabetes. Recently, cDNA clones encoding I-Aα and I-Aβ chains of NOD mice have been sequenced (408). The first external domain of the NOD I-Aβ chain was found to differ from that of MHC-matched (H-2^d) strains. A stretch of five nucleotide substitutions in the conserved region between positions 248 and 252 results in radical amino acid changes, particularly in residue 57 (Asp → Ser) of the NOD I-Aβ molecule—the same residue implicated in the DQβ chain, the human analog of the murine I-A chain, in Type I diabetes. These striking findings suggest that the variant murine and human MHC glycoproteins bind to a diabetogenic β cell antigen in similar ways.

Streptozotocin. Multiple injections of small doses of streptozotocin, a drug toxic for β cells, causes severe insulitis and diabetes in mice. The disease is preventable by antilymphocyte treatment (385). Additional evidence of an autoimmune component in this model is that the disease can be transferred by lymphocytes from diabetic mice to healthy recipients (385). Genes located in the H-2 complex determine differences in susceptibility to the disease in various mouse strains (410). The immune manifestations of streptozotocin-induced diabetes may be a consequence of the drug-induced β cell damage.

Autoimmune Diseases of the Thyroid

The human diseases

Hashimoto's thyroiditis is a disease affecting mainly middle-aged women. The disease is 20 times as frequent in women as in men. Its principle manifestation is swelling of the thyroid gland (goiter); in advanced cases there is underproduction of thyroid hormone. The resulting hypothyroidism leads to a variety of symptoms and signs, chief among which is sluggish metabolism and apathy. The gland is markedly infiltrated by T cells (CD4$^+$ and CD8$^+$), macrophages, and plasma cells, which together form secondary lymphoid follicles within the substance of the thyroid. Regeneration of thyroid follicles may occur in Hashimoto's disease, but in a related disorder, primary myxedema, the gland undergoes complete destruction. The CD4$^+$ helper T cells may become sensitized to some thyroid autoantigen due to unknown reasons. Alternatively, preexisting thyroid specific autoreactive T cells may become activated due to a lack of specific suppressor cells in genetically susceptible individuals (411). The CD4$^+$ T cells probably help B cells to produce autoantibodies against thyroid antigens. The serum of patients with Hashimoto's disease contains antibodies to thyroglobulin, the major iodine-containing protein within the acinar space of thyroid follicles (12). Autoantibodies to an approximately 107-kd protein in the cytoplasmic microsomal fraction of thyroid cells also occur in autoimmune thyroiditis (412). The microsomal autoantigen has been identified by cDNA cloning and sequencing to be a thyroid peroxidase (34,413). Although thyroglobulin and the intracellular microsomal proteins may seem sequestered from the immune system, there is some evidence that they are exposed on the surface of thyroid follicular cells in Hashimoto's disease (387). Destruction of thyroid epithelial cells may be brought about by complement-fixing IgG autoantibodies against thyroglobulin and thyroid microsomal antigens (387,411). In addition, natural cytotoxic T cells present in the infiltrate have been shown to kill thyrocytes by antibody-dependent cytotoxicity (411). Hashimoto's disease has a genetic basis. Multiple genes are involved, and there is an increased association with the HLA-DR5 haplotype. Moreover, asymptomatic family members have an increased incidence of thyroid autoantibodies and other endocrine organ specific autoantibodies (411).

Graves' disease, another autoimmune thyroid disease, has as its main manifestation overactivity of the thyroid gland (thyrotoxicosis), resulting in hyperactivity, tremulousness, and insomnia. Patients with this condition produce IgG autoantibodies against receptors for thyroid stimulating hormone (TSH) present on thyroid follicular (or epithelial) cells. The pituitary gland produces TSH, which in turn increases the production of thyroid hormone by the thyroid. The anti-receptor autoantibodies mimic the action of TSH and inappropriately stimulate the thyroid cells to secrete excessive amounts of thyroxine, a thyroid hormone (333). The thyroid in Graves' disease is infiltrated by lymphocytes. Autoreactive CD4$^+$ T helper

cells can be cloned from the infiltrate and they presumably help B cells to produce the autoantibodies (76,387). Treatment of Graves' disease with an antithyroid compound, methamazole (or treatment of Hashimoto's disease with thyroxine), leads to clinical remission *and* reduction in the autoantibodies of the disease (414). These agents may exert their effects by inhibiting the expression of thyroid autoantigens by thyrocytes or they may influence T cells (414).

Animal models of autoimmune thyroiditis

Obese strain chickens. Spontaneous autoimmune thyroiditis resembling Hashimoto's disease consistently develops in obese strain (OS) chickens. Hypothyroidism was first detected in a small percentage of female Cornell C strain (CS) chickens, from which the OS strain was developed by selective breeding (415). Both male and female OS chickens are equally affected. Hypothyroidism becomes overt in all OS chickens between 3 and 5 weeks of age due to destruction of the thyroid by mononuclear cells. The manifestations of the disease are small body size, large deposits of subcutaneous and abdominal fat (hence obese), lipemic serum, cold sensitivity, and infertility. There are high serum levels of anti-thyroglobulin antibodies in these birds (416,417), but unlike Hashimoto's thyroiditis there are no antibodies to thyroid microsomal antigens (416). That anti-thyroglobulin autoantibodies are one of the necessary factors for the development of the disease is shown by the failure of bursectomized chicks, which lack B cells, to develop thyroiditis (416). About 15% of the birds also produce autoantibodies to parietal cells of the stomach, just as in the human counterpart of autoimmune thyroiditis. In genetic studies with crosses between OS and normal CS chickens, anti-thyroglobulin autoantibodies were found in some progeny without thyroiditis, analogous to the asymptomatic family members of patients with Hashimoto's thyroiditis who also produce anti-thyroglobulin antibodies.

Activated Ia$^+$ T cells infiltrate thyroid gland of OS chickens, and they can transfer the disease to healthy recipients. Generalized abnormalities of the immune system are also evident: the ratio of helper/suppressor T cells is increased and there is hyperresponsiveness of peripheral blood or splenic lymphocytes to concanavalin A (416). Defects in the thyroid have been found before the onset of the autoimmune process. Degenerating thyroid epithelial cells and disturbed thyroid function are already present in newly OS hatched chicks (417). These abnormalities could be the consequence of damage by complement-fixing autoantibodies transferred from the OS mother to the embryo; such antibodies are found in the chick's thyroid follicular basement membrane (418). Nevertheless, some preexisting thyroid abnormality seems required for the disease to develop because the thyroid of normal chickens is resistent to destruction by anti-thyroglobulin antibodies (417). The development of thyroiditis in OS chickens is under polygenic control; MHC genes are important, but non-MHC genes also play a role (416).

Experimental autoimmune thyroiditis. Injection of thyroglobulin with adjuvants leads to the development of autoantibodies to thyroglobulin and an inflammatory thyroiditis resembling Hashimoto's disease in several animal species. The autoantigen, an iodinated glycoprotein of 660-kd molecular mass, is stored in the colloidal space of thyroid follicles, but small amounts of it are also present in the circulation. A 5- to 10-kd tryptic fragment of thyroglobulin has been shown to induce thyroiditis in CBA mice (419). This peptide appears to contain the dominant T cell epitope required for inducing proliferation of thyroglobulin-reactive helper T cells. The degree of iodination of thyroglobulin may also be important in inducing T cells that react to the autoantigen (420). The immunologic and genetic factors involved in the development of the disease have been dissected in inbred mouse strains (421). MHC genes have a prominent role. Genes located in the I-A subregion determine the extent of both autoantibody production and pathological lesions. In addition, genes at the D end of MHC influence the development of effector T cells. Mouse strains with H-2 haplotypes *k*, *s*, and *q* are high responders to mouse thyroglobulin, and develop severe thyroiditis, whereas mice with H-2 *b*, *d*, and *j* haplotypes are low responders and develop little or no thyroiditis (421). T cells play a central role. Normal mouse strains have T helper cells that can recognize unaltered syngeneic thyroglobulin and that can initiate autoimmune thyroiditis. Concomitantly, T suppressor cells that can prevent the proliferation of self-thyroglobulin-reactive T helper cells are also naturally present in normal mouse strains. In high-responder strains, the balance is favored toward the helper cells. Both types of T cells can be cloned from normal mice (420,422,423), and anti-thyroglobulin helper T cell lines can produce thyroiditis in H-2 syngeneic mice (420,422,423).

Autoimmune Diseases of the Central Nervous System

The human disease

Inflammation of the central nervous system (CNS) can complicate certain viral infections and, in rare cases, antimicrobial vaccination. In these instances of postinfectious or postvaccinal encephalomyelitis, mononuclear cells infiltrate the CNS, destroying brain cells (astrocytes and oligodendrocytes). Loss of the myelin sheath of nerve cells (demyelination) occurs, resulting in neurological symptoms that culminate in coma or paralysis. The lesions may be mediated by T cells that become sensitized to autoantigens present in the white matter of the CNS, particularly myelin basic protein. T cells specific for myelin basic protein can be cloned from the spinal fluid of patients with postinfectious encephalomyelitis (424). The viruses that initiate the autoimmune response to myelin basis protein need not be present at the site of inflammation or at the time overt disease develops. The viral infection may trigger the production of antibodies and T

cells that cross-react with myelin basic protein, by molecular mimicry.

Multiple sclerosis is a chronic, often relapsing disease of considerable medical importance. It is the commonest demyelinating disease of the brain and spinal cord. The disorder affects males and females equally and usually begins between the ages of 20 and 40. Multiple sclerosis can have a subtle onset, but its course is usually progressive and in some patients relentless. The introduction of nuclear magnetic resonance imaging of the brain and spinal cord has greatly assisted the diagnosis. The abnormal images obtained with this technique are due to localized demyelination of white matter, which results in characteristic plaques. Microscopically, there are infiltrates of T cells (both helper and cytotoxic) and macrophages in the brain (425). However, T cells reactive to myelin basic protein or other CNS proteins have not been consistently found in multiple sclerosis; most of the T cells in the spinal fluid are autoreactive, proliferating to self-MHC antigens (424). The spinal fluid also contains increased amounts of immunoglobulins, which are often oligoclonal (426). Viral infections have been implicated, but there is no conclusive proof for a viral etiology of the disease; the latest, but controversial, etiologic candidate is a retrovirus (427,428).

Experimental allergic encephalomyelitis (EAE)

An acute inflammatory disease of the CNS can be induced by a single injection of brain or spinal cord tissue with adjuvant in different laboratory animals—from rodents to primates. The same disease occurs whether brain tissue or myelin basic protein is injected. In mice and rats, paralysis of the hind legs begins 2 to 3 weeks after the challenge. Within the brain, perivascular infiltration of inflammatory cells precedes the clinical signs. Although antibodies to myelin basic protein are produced, EAE is T cell mediated. Transfer of myelin basic protein specific T cells that are sensitized *in vitro* or *in vivo* (from animals with EAE) into normal recipients can induce the disease (50,429,430). The sensitized T cells are I-A restricted and CD4$^+$, and they initiate the inflammatory lesions by recruiting both CD4$^+$, and CD8$^+$ effector cells to the brain (50,429,430). Glial cells (astrocytes and oligodendrocytes) express myelin basic protein on their surface and are induced to express high levels of Class I and Class II MHC antigens by lymphokines produced by the infiltrating T cells and macrophages (425,429,430). Endothelial cells of blood vessels in the brain also express increased levels of Ia (81,82). These events perpetuate the inflammation and demyelination. Treatment of mice with anti-Ia or anti-L3T4 antibodies can prevent the induction of EAE and reverse overt disease (431).

Susceptibility to EAE is genetically controlled. In guinea pigs, the sensitivity is inherited as a dominant trait linked to the MHC of strain 13, although another gene not linked to MHC is necessary. In mice the situation is more complex. SJL (H-2^s) mice are partially susceptible and BALB/c mice (H-2^d) are resistant, whereas (SJL ×

BALB/c)F$_1$ mice are fully susceptible (429,430). Genes outside the MHC complex may therefore be important in determining susceptibility of mice to EAE. The resistant BALB/c strain can be rendered susceptible by low doses of cyclophosphamide or irradiation, thus indirectly implicating suppressor T cells in the process.

Myelin basic protein from various species has been characterized. Its molecular weight is 17 to 18 kd, and it consists of 170 amino acid residues. Many sites in the molecule contain immunogenic epitopes, but only some are encephalitogenic. In the case of bovine or human myelin basic protein, peptides spanning residues 114 to 122 are encephalitogenic in guinea pigs but not in rabbits; the encephalitogenic site for rabbits consists of 10 amino acids located between residues 66 and 75 of rabbit myelin basic protein. In PL/J mice (H-2^u) the amino terminal residues (1–11) of myelin basic protein contain the encephalitogenic epitope, whereas in SJL/J mice (H-2^s) the epitope resides in position 89–101. Differences in Class II molecules, and consequently their ability to present only certain peptides to autologous T cells, as well as the use of different T cell receptors, can account for their distinct encephalitogenic determinants (430a). Only T cells that are specific for the encephalitogenic sites of myelin basic protein can transfer the disease to normal recipients.

Suppression or prevention of EAE can be achieved by injecting, without adjuvant, the same myelin basic protein that induces disease, or by injecting peptides containing the nonencephalitogenic sites of the protein (50,429). Moreover, replacement of the single tryptophan residue with tyrosine in the encephalitogenic peptide of myelin basic protein can also suppress EAE in guinea pigs (50). A synthetic basic copolymer (Cop1), composed of L-alanine, L-glutamic acid, L-lysine, and L-tryosine, inhibits the induction of EAE in animals subsequently challenged with myelin basic protein in adjuvant (432). The inhibition is specific: neither the D-amino acid copolymer nor acidic copolymers prevent EAE. A good correlation exists between the suppressive activity of various synthetic copolymers and their immunological cross-reactivity with the encephalitogenic sites of myelin basic protein at the T cell level. The mechanism of inhibition of EAE hinges on the induction of specific suppressor T cells: it can be transferred by cyclophosphamide-sensitive T cells (429,432). A clinical trial of the efficacy of Cop1 in multiple sclerosis has shown promising results (433).

Encephalitogenic T helper cell lines specific for myelin basic protein, attenuated by irradiation, can also induce resistance to EAE and generate specific T suppressor cells when inoculated into syngeneic recipients. The suppressor T cells are specific for idiotypes (i.e., they are anti-idiotypic) on the clonotypic receptors of the encephalitogenic T cells (312). Thus in this model we also see a balance between suppressor T cells and helper T cells that modulates susceptibility or resistance to an autoimmune disease. Several recent studies have shown that myelin basic protein-specific T cells in EAE use a restricted set of T cell receptor genes and that the disease can be prevented with the corresponding anti-T cell receptor antibodies (434,434a).

Myasthenia Gravis

The human disease

Myasthenia gravis is a disorder of neuromuscular transmission in which there are autoantibodies against the acetylcholine receptors of neuromuscular junctions. The disease can be transferred through the placenta from mothers to infants, presumably by the autoantibodies (435). Females are affected twice as often as males, typically during the third decade of life. Muscular weakness, the predominant feature of the disease, classically involves the ocular, pharyngeal, laryngeal, and respiratory muscles. Clinical signs include drooping of the eyelids, double vision, choking on food, and a peculiar "nasal" voice. Weakness of the arms and legs can occur in advanced cases, but it is atypical. There is an association between myasthenia gravis and hyperthyroidism. The thymus is also important in myasthenia gravis. Thymomas occur in about 10% of patients, but even more interesting is the curative effect of thymectomy, which can be dramatic in young patients with the disease (436).

The acetylcholine receptor consists of five subunits, two α and one each of β, γ, and δ, but only a small region of the receptor, formed by its α subunits, binds to acetylcholine. This region is the immunodominant portion of the receptor (437). Only a minor population of myasthenia autoantibodies is specific for the actual acetylcholine binding site of the receptor (437). The fact that α-bungarotoxin, a readily purified venom, binds to the receptor has been exploited experimentally: myasthenia autoantibodies block the binding of α-bungarotoxin to the receptor (438). However, this effect is probably due to steric hindrance because the toxin has a MW of 8,000—much larger than the receptor's natural ligand, acetylcholine (134). The autoantibodies are of the IgG class and they have a high affinity for the receptor. Their production is T cell dependent. There is evidence that cross-linking by the autoantibodies leads to endocytosis of the receptors. Complement-mediated focal lysis may also account for loss of receptors. However, blocking antibodies are probably the most important types of autoantibodies in the disease; they occur in 90% of patients with myasthenia gravis.

Musclelike myoid cells containing acetylcholine receptors are present in the normal thymus, so it is possible that the autoimmune response may originate in this organ (77,79). Thymuses removed from patients with myasthenia gravis contain germinal centers of the type found in antigen-stimulated lymph nodes. Helper T cells specific for the acetylcholine receptor and B cells producing anti-receptor autoantibodies have been isolated from the thymuses and blood of myasthenic patients (78,79,439). MHC-restricted T cell lines that respond to the receptor and that help B cells to produce anti-receptor antibodies in vitro have been characterized (78): the majority of the T helper cells recognize an epitope located on a denatured (processed?) α subunit of the receptor, whereas the autoantibodies recognize a conformational determinant on the α subunit.

Animal models

Experimentally induced myasthenia gravis has been extensively studied in Lewis rats and inbred strains of mice. A single injection of acetylcholine receptor, purified from the electric organs of the eel *T. californica*, along with adjuvants, causes an acute phase of weakness within 8 to 12 days and then chronic weakness after about 30 days. The response to the eel receptor is T cell dependent (440), and *in vitro* T cell responsiveness correlates with both the antibody response and susceptibility to the disease (440). In mice, I-A subregion genes are important (441). The C57BL/6 strain (H-2^b) is a high responder to *Torpedo* receptor and highly susceptible to myasthenia. By contrast, mutant B6.C-H-2^{bm12} mice, which differ from C57BL/6 mice only by a limited region in the external domain in the A-β chain of Ia, are low responders and resistant to the disease (441).

Pemphigus

Pemphigus (from the Greek *pemphix*, a blister) is a bullous disease of the skin and mucous membranes. There are several varieties of pemphigus, the commonest of which is pemphigus vulgaris (Table 12). The immediate cause of the blisters is *acantholysis*, a loss of cohesion between the epidermal cells of the skin due to disappearance of intercellular bridges. The acantholytic lesion may contain an inflammatory exudate, but a more characteristic feature in all types of pemphigus lesions is the presence of autoantibodies against the intercellular cement substance that holds the epidermal cells of the skin together (Fig. 16). The serum also contains such autoantibodies, and they tend to fluctuate in titer concordantly with the activity of the disease (442). An unusual triad of pemphigus, myasthenia gravis, and thymoma has been observed (443), and there is evidence that pemphigus autoantibodies can bind to Hassal's corpuscles within the thymus (444). Interestingly, canine pemphigus is also associated with thymoma (445). Another variety of pemphigus, the Senear–Usher syndrome, has certain features of SLE (446).

Pemphigus antibodies bind to stratified squamous epithelium of all mammmals and birds (447), but the chemical properties of these conserved autoantigens are not entirely clear. Proteins of 50, 66, 160, and 210 kd, from different sources, have been shown to bind to pemphigus autoantibodies (442,448). The latter two glycoproteins represent distinct autoantigens: the 210-kd glycoprotein, consisting of two chains (80 and 130 kd), is present in the lower region of the epidermis, the site of involvement in pemphigus vulgaris, and it binds to antibodies from patients with pemphigus vulgaris. The 160-kd antigen, identified by pemphigus foliaceus antibodies, is a glycoprotein (desmoglein I) in the upper region of the epidermis, the region involved in pemphigus foliaceus (449).

IgG pemphigus antibodies cause acantholysis of cultured human skin within 2 to 3 days; complement is not required for the effect (450) but its presence greatly mag-

TABLE 12. *Clinical and immunological features of pemphigus*

Disease	Clinical features	Autoantibodies
Pemphigus vulgaris	Oral and cutaneous bullae	Intercellular IgG
Pemphigus vegetans	Oral lesions and wartlike rash	Intercellular IgG
Pemphigus foliaceus	Superficial skin blisters	High epidermal IgG
Senear–Usher syndrome	Mimics facial SLE	Dermal–epidermal IgG
Brazilian pemphigus	Superficial skin blisters	High epidermal IgG

nifies the extent of the *in vitro* lesion (451). In addition to acantholysis, the autoantibodies also cause epidermial cells to release plasminogen activator (452). This proteolytic enzyme is thought to participate in the mechanism that causes the loss of adhesion between epidermal cells, perhaps by degrading the intercellular cement substance (442). Pemphigus vulgaris autoantibodies also cause acantholysis when injected into animals. This has been shown in two ways: the induction of acantholysis in human oral mucosa transplanted into nude mice (453) and reproduction of the bullous lesion by human pemphigus antibodies injected into newborn mice (305,454). Mice deficient in C5 can develop the lesion when injected with pemphigus autoantibodies, thereby indicating that complement is not required for the formation of bullae (451). A dramatic demonstration of the pathogenic activity of pemphigus autoantibodies is the development of the disease in the infant of a mother with active pemphigus vulgaris. In such cases pemphigus autoantibodies have been detected in the skin lesions and blood of the baby, and the bullae wane as the maternal autoantibodies are catabolized (305,454). MHC genes are also important in the development of pemphigus vulgaris. DR4-DW10 and DQw1.9 alleles are associated with susceptibility to the disease (374,397).

SUMMARY AND CONCLUSIONS

In this chapter we have discussed the highlights of past accomplishments in the field of autoimmunity, and we have pointed out some of the major directions of new research on this topic. Out of necessity, a wealth of detail has been omitted. Even so, readers of this chapter should have learned these main points.

1. The capacity to produce autoantibodies is an inherent property of the normal immune system; the vast immunoglobulin repertoire gives B cells the potential to pro-
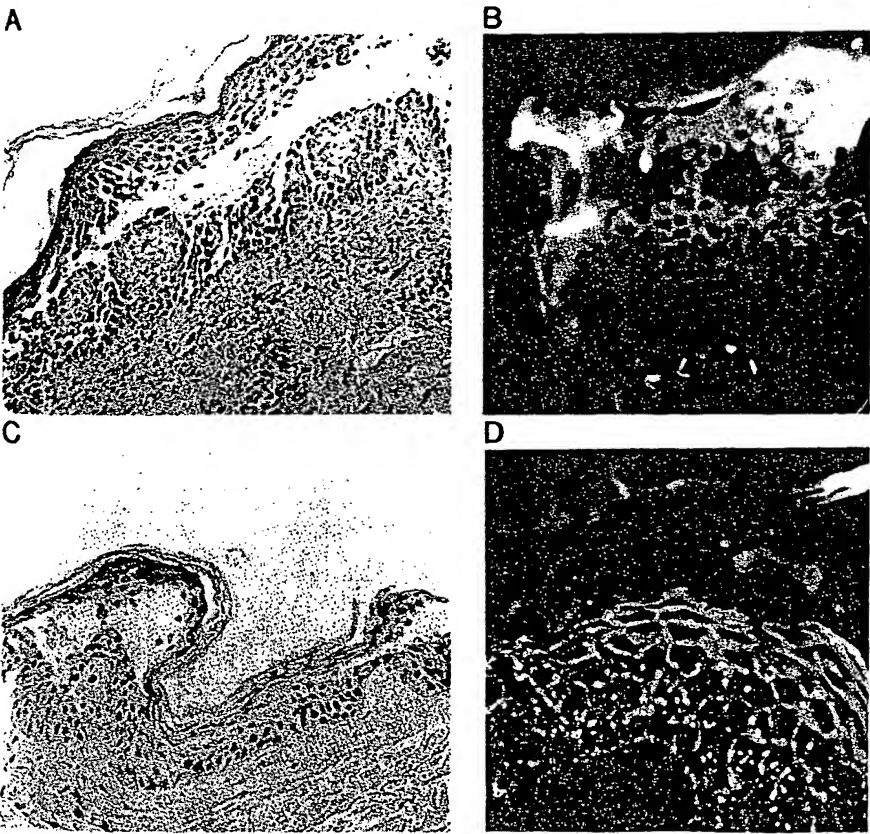

FIG. 16. Immunopathology of pemphigus. **A** and **C:** Histologic sections showing acantholysis and the formation of bullae in pemphigus vulgaris (A) and pemphigus foleaceus (C). **B:** Immunofluorescence microscopy showing deposition of IgG in the intercellular cement substance within the lower epidermis of a lesion of pemphigus vulgaris. Note the acantholysis. **D:** IgG deposits in a lesion of pemphigus foleaceus. The intercellular cement substance of the upper layer of the epidermis is coated with IgG. These photographs were obtained through the generosity of Dr. Ernst Beutner, Immunodermatology Unit, Department of Microbiology, State University of New York at Buffalo.

duce autoantibodies against a seemingly inexhaustible list of autoantigens.

2. Nevertheless, autoantibodies, including natural autoantibodies, are virtually never formed against certain autoantigens such as those of the ABO blood group system. These cases probably entail deletion of particular autoreactive B cell and T cell clones early in ontogeny.

3. By contast, numerous other kinds of autoantibodies are produced normally, but only in small amounts and only as low-affinity IgM antibodies. It is highly likely that these innocuous natural autoantibodies are encoded by unmutated germline V genes.

4. Autoimmunity, a normal process, becomes a pathological event (an autoimmune disease) with the production of large amounts of certain kinds of IgM autoantibodies (e.g., cold agglutinins) or of particular kinds of high-affinity IgG autoantibodies. The latter process requires T cells.

5. T cells with the capacity to respond to organ specific autoantigens are also present in the normal immune repertoire. They have the capacity to produce lesions if the T cell compartment is destabilized, a pathogenic mechanism that presumably involves loss of antigen specific suppressor cells.

6. All autoimmune diseases are divisible into two families: organ specific and systemic. The pathogenic mechanisms that provoke these two classes of immunologic diseases are different. Highly relevant to organ specific autoimmunization are tolerance and suppression within the T cell compartment, aberrant expression of MHC antigens, antigenic mimicry, and allelic variations in MHC genes. The pathogenesis of systemic autoimmune diseases probably involves polyclonal B cell activation, as well as abnormalities of immunoregulatory T cells, T cell receptor and MHC genes, and idiotypic networks.

7. There is no example of the induction of a systemic autoimmune disease by immunization of experimental animal with an autoantigen. By contrast, numerous organs of the body are susceptible to an autoimmune attack engendered by immunization with organ specific autoantigens. The susceptibility of normal animals to T-cell-dependent autoimmunization by organ specific autoantigens supports the concept that the normal immune repertoire harbors a wide spectrum of antiself T cells.

8. The occurrence of autoantibodies in a disease does not consistute proof that the disease has its basis in autoimmunity. Nor does their presence imply their pathogenicity.

9. Susceptibility to autoimmunization and the development of autoimmune diseases are genetically controlled, not only by genes within the MHC complex but also by other loci, most of which are unidentified, elsewhere in the genome. The development of an autoimmune disease probably entails the interaction of genetically controlled mechanisms with the environment.

Readers of this chapter should recognize by now that studies of autoimmunity and autoimmune diseases, along with the rest of research in immunology, have entered a new era. The conversion of autoimmunity research from seropathology to molecular biology emphasizes that a so-

lution to the problem of autoimmunization is inextricably tied to our understanding of how the *normal* immune system operates—and vice versa. We noted at the beginning of this chapter that research in autoimmunity has gone through three revolutions. The fourth, it is our belief, will occur by reconciliation of the present advances in molecular immunology with actual clinical events. Only then will we have a full understanding, and perhaps novel and precise forms of therapy, of autoimmune diseases.

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